Timing of oviposition by western flower thrips (Thysanoptera: Thripidae) in apple fruit

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ABSTRACT

Adult western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), were most abundant on flower clusters of apple, *Malus* × *domestica* Borkhausen, from king bloom to full bloom. Low numbers of thrips remained on the clusters after petal fall as fruit enlarged. Thrips larvae peaked in numbers after densities of adults had peaked, usually by petal fall. Two staining procedures were developed for detecting thrips eggs in the surface of fruit ovary tissues (the edible portion of fruit), and in other blossom tissues (stamen, style, calyx, stem and leaves). Eggs were abundant in the latter tissues throughout the bloom and post-bloom periods; the calyx appeared to be highly preferred. Few eggs were detected in fruit ovary tissues during bloom. Egg numbers in ovary tissues began to increase about 8-13 d after full bloom, when fruit had grown beyond 5 mm diameter. The most effective timing of pesticides corroborated the oviposition data. Formetanate hydrochloride or spinosad caused the greatest reduction in oviposition injury (pansy spot) when applied from full bloom to about 5 mm fruit diameter.

Key Words: *Frankliniella occidentalis*, western flower thrips, pansy spot, oviposition, apples, sampling

INTRODUCTION

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), damages the fruit of a number of crops (Childers 1997). Feeding by larvae or oviposition by adult females produces distinct symptoms which can occur at different times during fruit development. Feeding on peach fruit after bloom causes a superficial skin blemish referred to as 'silvering'. Nectarines develop a more serious skin deformity from feeding called 'russetting' (Childers 1997). Damage on a number of other crops is caused by oviposition. The disorder known as pansy spot of apple, *Malus* × *domestica* Borkhausen, a pale halo surrounding a corky, raised scar, is caused by oviposition of female thrips (Newcomer 1921). Pansy spot marks are most visible on light-coloured fruit and result in downgraded fruit quality (Madsen and Proctor 1982). Damage on grapes is identical to pansy spot of apples (Jensen 1973), and a similar pale halo surrounds oviposition scars on tomato (Salguero Navas *et al.* 1991).

The timing of thrips oviposition on apple fruit is still a matter of dispute. Newcomer (1921) was first to discover thrips eggs in the center of pansy spots. He deduced that oviposition must occur "some time during bloom", and advocated an insecticide application at pink (opening flower buds) to control the adults responsible. Venables (1925) believed that oviposition in fruit occurred "some time between bloom and closing of the calyx", which occurs shortly after petal fall (80% of the flowers with no petals). Childs (1927) observed oviposition in fruit occurring much later, shortly after bloom and continuing

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until fruit were about 25-38 mm diameter. Madsen and coauthors (Madsen and Jack 1966, Madsen and Arrand 1971, Madsen and Proctor 1982) believed that overwintering adult female *F. occidentalis* first lay eggs on sepals and flower parts, and not on the edible portions of young fruit, beginning in early bloom. The larvae develop in the corolla and pupate away from the flower clusters. New adults begin emerging after petal fall and it is these individuals that lay eggs in the developing fruit. However, because western flower thrips pupates in soil litter, these newly emerged adults would have to recolonize the apple clusters to be responsible for the damage, and it is not yet clear that this recolonization occurs to any extent. The detailed work by some recent authors (Terry and DeGrandi Hoffman 1988, Terry 1991) concluded that oviposition on young fruit occurs during pink to full bloom (80% of the flowers fully in bloom), although the majority of eggs deposited during those stages are laid in flower parts, and therefore do not cause injury.

The current control recommendation is to apply insecticides either at pink (Smith *et al.* 2005) or during bloom, when adult western flower thrips are most abundant (Terry and DeGrandi Hoffman 1988). Insecticides (formetanate hydrochloride, spinosad, and acetamiprid) used for control of thrips on apple, are moderately toxic to bees; materials that are highly toxic to bees are restricted during bloom. Mitigation of toxicity is accomplished by spraying either at night or in the early morning before bees are actively foraging. Targeting sprays after bloom would expand the choice of chemical control tactics, and eliminate hazards to pollinators, providing a benefit to apple pest management.

The purpose of this investigation is to assess the timing of thrips oviposition on apple fruit in central Washington, and evaluate insecticide timing based on this information. We have built on the work of previous authors by expanding the time period and tissues in which the eggs are observed, as well as testing our data with insecticide timing experiments.

MATERIALS AND METHODS

Timing of Oviposition in Fruit. Densities of eggs, larvae, and adult thrips on apple were assessed in 2004-2006 at three locations. In 2004, a block (about 2 ha) of 'Delicious' trees at the Washington State University Tree Fruit Research and Extension Center (WSU-TFREC) in Wenatchee, WA, was used. No chemical thinning sprays were used in the block. Blossom clusters (n=100) or, starting at petal fall, king fruit (n=100) were collected at intervals corresponding to the developmental stages of the apple bloom. One sample was taken per tree. Sample timings were early tight cluster (flower buds unopened and touching) (2 and 6 April), pink (9 April), king bloom (bloom of the first, central flower in the blossom clusters) (13 April), full bloom (14 April), the start of petal fall (19 April), and four stages of fruit growth (23 April, 27 April, 4 May, and 11 May). Plant tissue samples were placed in self-sealing plastic bags, stored at 5 °C and processed within a week. Thrips were separated from plant tissues by filling the bag with water, adding a few drops of liquid detergent, and agitating for several seconds. Thrips and plant material were separated from the soapy water by pouring through two nested sieves (Hubbard Scientific Co., Northbrook, IL). The larger sieve (#10, 0.25 mm mesh) trapped most of the plant material, and the finer sieve (#230, 0.0014 mm mesh) trapped the thrips (Lewis 1997). Thrips were then rinsed into a vial of 50% ethanol. Adults and larvae were recorded separately.

After the plant tissue samples were washed, blossom clusters were trimmed so that only the king bloom fruitlet ovary tissues remained, and these were examined for thrips eggs. Only eggs in this structure, which becomes the edible portion of the fruit, cause economic damage. Direct obser-

vation of apple fruit, a method used by Terry (1991), was found unsatisfactory. A staining technique (Backus *et al.* 1988, Teulon and Cameron 1995) was used instead. Trimmed fruitlets were placed in McBride's stain (0.2% acid fuchsin in 95% ethanol and glacial acetic acid (1:1 vol/vol)) for 24 h, then transferred to clearing solution consisting of one part each of distilled water, 99% glycerin, and 85% lactic acid (1:1:1 vol/vol/vol). This method allowed samples to be stored for up to three months without deterioration. Samples in the clearing solution were heated in a double boiler under a fume hood for 1 h to soften the tissue. After clearing, the skin and underlying flesh of the fruit was sliced off to a thickness of 0.5 mm, and the remaining fruit tissue discarded. The skin was placed between two glass microscope slides and pressed flat. The thin tissue was observed under a dissecting microscope using transillumination to reveal the darker eggs.

The timing of egg deposition was further studied in 2005 in a commercial orchard block (2 ha) of 'Braeburn' apples near the town of Omak, WA, and in 2006 in a commercial orchard block (4 ha) of 'Cameo' apples in Bridgeport, WA. Flower clusters and king fruit were sampled as described previously, including sample sizes. In 2005, samples were taken at tight cluster (16 April), pink (20 April), king bloom (24 April), full bloom (28 April), petal fall (1 May), and five stages of fruit growth (4, 8, 11 16 and 23 May). In 2006 samples were taken at pink (24 April), king bloom (28 May), full bloom (4 May), the end of petal fall (12 May), and four stages of fruit growth (16 and 24 May, 2 and 9 June). The larger range of fruit sizes collected in 2006 necessitated slightly modified handling. Smaller fruit (<15 mm diameter) were stained and examined as described previously. Larger fruit (≥15 mm) were stained only, without clearing or dissection. Because the skin of the larger fruit was almost free of trichomes, the punctures and pink-stained eggs were clearly visible using light microscopy. In a few rare cases, oviposition sites were excised for closer examination.

Apple Tissue Oviposition Studies. In addition to studies of oviposition in apple fruit ovary tissues, more detailed studies were carried out to determine relative oviposition preference in both vegetative and reproductive tissues of apple. Blossom clusters were collected from three orchards located in Yakima County, WA in both 2005 and 2006. Apple varieties included were 'Delicious', 'Fuji', and 'Granny Smith'. At each orchard, a sample consisted of 10 blossom clusters, randomly selected from several trees along the orchard edge, shown in another study (Miliczky *et al.* 2007) to support the highest densities of ovipositing thrips. In 2005, clusters were collected at five developmental stages: pink, king bloom, full bloom, petal fall, and 15 - 25 mm fruit size. In 2006, the pink through petal fall stages were again sampled, but two post-petal fall samples were taken: 10 mm fruit size and 25 mm fruit size. Clusters were placed in self-sealing plastic bags, and transported to the laboratory on ice.

A modified egg staining procedure was used for these studies. Blossom samples were immersed in a warm (60 °C) solution of white vinegar (Heinz Distilled White Vinegar, H.J. Heinz, Pittsburgh, PA) and blue food colouring (McCormick Blue Food Coloring, Hunt Valley, MD), using six drops of food colouring in 40 ml of vinegar. Samples were left in the solution for 20 min, after which the tissues were removed from the solution and blotted dry. The vinegar caused tissue layers to separate, exposing the eggs, while the blue food colouring stained the oviposition scar and egg. Samples were examined under a dissecting microscope to assess egg numbers.

Egg numbers on each of the five structures were determined: flower reproductive organs (stamen, style), flower calyx, stem below flower or fruitlet, leaves, or fruitlet ovary tissues. For each blossom cluster, we counted total number of leaves and flowers in the cluster, and then randomly selected a subsample of three flowers and three leaves of each cluster for examination. Once egg numbers had been determined for each of the five structures within this subsample,

we used these estimates of density in combination with our counts of leaves and flowers in the cluster to obtain by extrapolation an estimate of egg numbers for each of the five structures in the entire blossom cluster. Percentage distribution of eggs among the five structures was then determined using these extrapolated estimates of egg densities. Samples from the three orchards were pooled.

2004 Insecticide Timing Trial. This trial was conducted at the same site used for the 2004 oviposition study. Insecticides were used to kill adult thrips in blossom clusters at different stages of blossom or fruit development. One hundred 'Delicious' apple trees were selected randomly, and one branch with 7-10 flower clusters was selected on each tree. Adult thrips were allowed to migrate back to the branches, thus treatments created periods of reduced adult presence. The experiment was a completely randomized design with 10 treatments (spray timings) and 10 replicates (branches). Thrips were killed by spraying the branches to drip with a solution of formetanate hydrochloride (Carzol® 92SP, Gowan Co., Yuma, AZ) at 27.6 g AI/100 litres. Material was mixed in a ½-litre spray bottle. Applications were made at one of nine stages from tight cluster through about 16 mm fruit diameter (Table 1). One group of 10 branches was sprayed on all nine dates to eliminate thrips during the entire bloom and early fruit growth period. Fruit were harvested from each treatment on 24-25 May. The proportion (p) of fruit injured by thrips was transformed by arcsine [square root (p)], then analyzed using analysis of variance for a completely randomized design (Statistical Analysis Institute 1988). Treatment means were separated with a Least Significant Difference test, α=0.05.

2006 Insecticide Timing Trial. This experiment was conducted in the same commercial orchard block used for the 2006 oviposition study. The orchard (4 ha) was bordered on three sides by native vegetation. The 8-year-old 'Cameo' trees were pruned to a trellis about 3 m high. Tree spacing was 0.9 × 3.7 m. The experiment was a randomized complete block design with blocks determined by proximity to the native vegetation. Four replicated plots (15 trees in a single row) were sprayed with either formetanate hydrochloride or spinosad (Success® 2SC, Dow AgroSciences, Indianapolis, IN) using an airblast orchard sprayer (Rears Pak-Blast, Rears Mfg, Eugene, OR) calibrated to deliver 1,871 litres/ha. Plots were separated by five untreated rows. Application timings ranged from king bloom through 27 mm fruit size (Table 2), plus an untreated check. The first three sprays were applied at night to avoid contact with pollinators. All other sprays were applied during the day.

Between 110 and 150 fruit per plot were examined for pansy spot under a microscope on 6 June. The proportion (p) of fruit with pansy spot was transformed with arcsine [square root (p)]. Data were analyzed with two-way ANOVA for two insecticide treatments and six application timings. Main effects means for insecticide or spray timing were separated with a Least Significant Difference test.

RESULTS AND DISCUSSION

Timing of Oviposition in Fruit. The abundance of adult thrips on apple blossom clusters increased as apple blossoms opened (Fig. 1). Abundance in blossoms was highest at king bloom. From petal fall onwards, king fruit were sampled rather than blossom clusters. Adults were most abundant on king fruit at the beginning of petal fall, then decreased rapidly. Low numbers remained in the samples during the post petal-fall period in 2004 and 2006. Adults were still recovered on the 17.3 mm diameter fruit sample date in 2006, 24 d after full bloom. Larvae, which could not be identified to species, peaked after adults, usually on king fruit after petal fall (Fig. 1). Contrary to expectation, the peak abundance of eggs in fruit was later than the peak in larvae, with

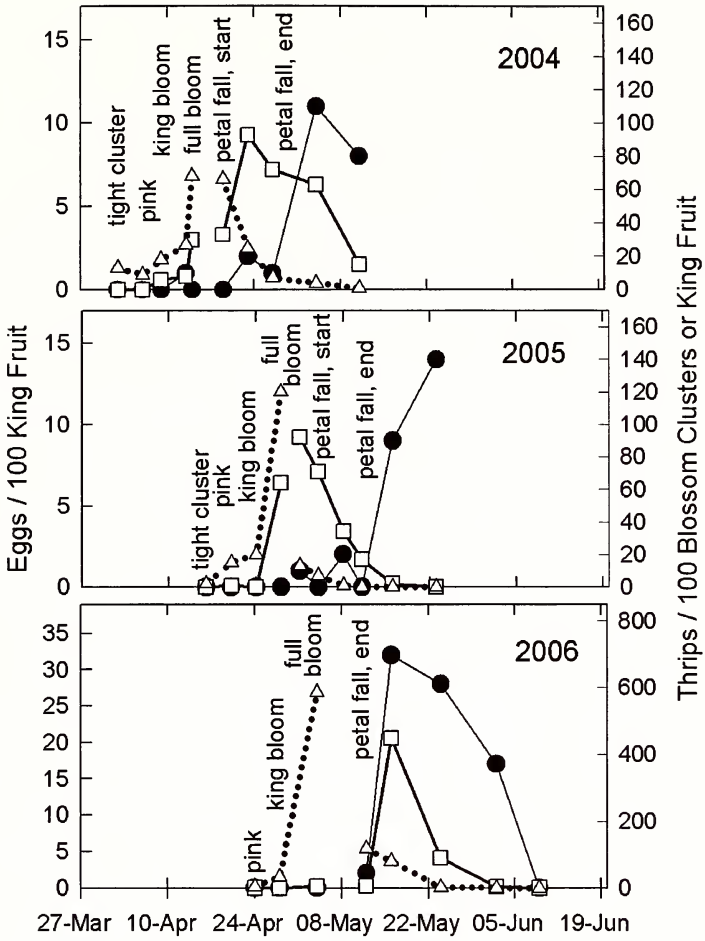

Figure 1. Densities of thrips eggs in king fruit ovary tissues, and adult and larval stages on blossom clusters (tight cluster to full bloom) and king fruit (petal fall and later). Adult thrips (Δ); larvae (□); eggs (●).

a relatively long lag period between peak abundance of adults in flower clusters and eggs in the fruit ovary tissues. This result suggests that females deposited eggs extensively in other, unsampled plant structures within the blossom clusters (see Apple Tissue Oviposition Studies).

Relatively few thrips eggs were found in fruit during the period most often targeted for sprays (Fig. 2), viz., pink through petal fall, although this is the period when adults were most abundant (Fig. 1). The greatest increase in egg numbers deposited in fruit occurred after the start of petal fall, between 5.6 - 9.0, 6.0 - 11.6, and 5.7 - 12.5 mm diameter in the three years of the study. This corresponded with 13 - 20, 13 - 18, and 8 - 16 d after full bloom, respectively. This is substantially later than is reported by some authors (Newcomer 1921, Venables 1925, Terry 1991), but in general agreement with others (Childs 1927, Madsen and Jack 1966). Stained eggs were detected in oviposition scars after 25 mm in 2006 but were no longer found in scars in fruit that were 31.7 mm diameter in early June, which agrees with the observations of Childs (1927).

Apple Tissue Oviposition Studies. The calyx and stem received between 72 and 98% of the eggs deposited in sampled blossom clusters, depending upon blossom stage (Fig. 3). By the time fruit reached approximately 10 mm in size, they had become moderately attractive as oviposition sites. Eggs in fruit ovary tissues never ex-

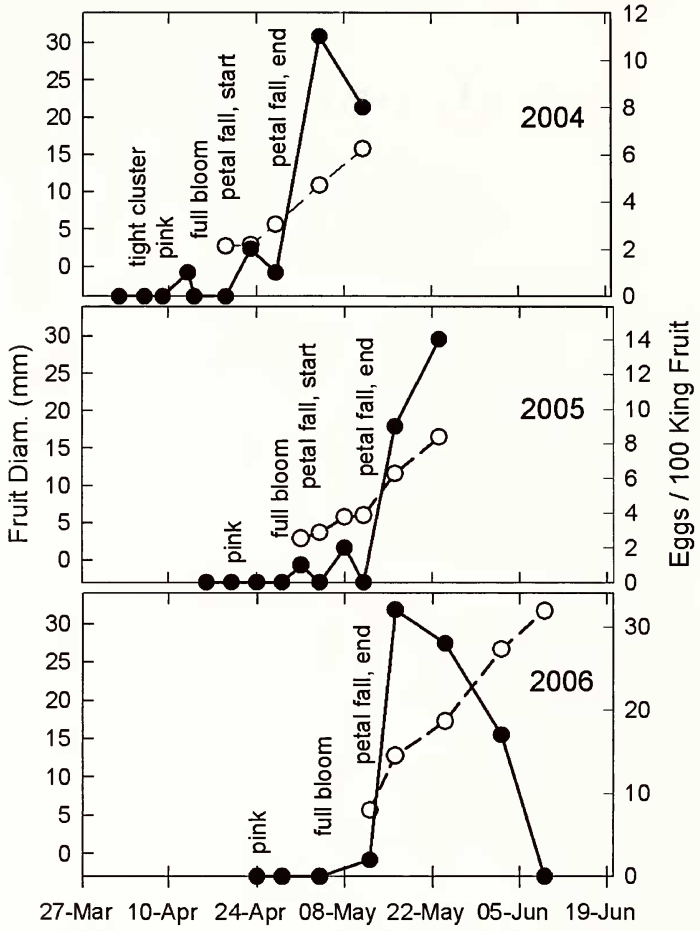

Figure 2. Thrips egg densities in king fruit ovary tissues (•) and king fruit size (○).

ceeded 13% of the total eggs found at any blossom stage. Some oviposition also occurred in leaves and floral reproductive parts (stamens and styles), but this comprised a small proportion of the total eggs.

This study provides insight into several issues regarding thrips damage to apple. In the fruit oviposition timing studies, where only eggs laid in fruit ovary tissues were assessed, the larvae appeared to be out of sequence with the eggs (Fig. 1). The larvae may have been hatching from eggs laid in structures not monitored during that study. The second issue deals with predicting the amount of fruit damage from adult density estimates. The high percentage of eggs being laid in non-fruit tissues may be responsible for the overall poor correlation between densities of adult flower thrips and fruit damage in apples (Terry 1991, Bradley and Mayer 1994).

2004 Insecticide Timing Trial. The timing of formetanate hydrochloride applications affected the percentage of fruit damaged ($F = 4.45$; df = 9,96; $P < 0.0001$). Applications were most effective in reducing pansy spot during the period from the start of petal fall to 5.6 mm fruit; applications before or after this timing were less effective (Table 1). An application at king bloom, for example, was clearly too early. The optimum timing was, as would be expected, just before the peak oviposition that occurred some time between 5.6 and 10.9 mm fruit size (Fig. 2). The results indicated that prevention of the oviposition surge at the end of petal fall was key for pest management, but that the entire period of oviposition was not of interest. In fact, one well-timed spray at petal fall was equiva-

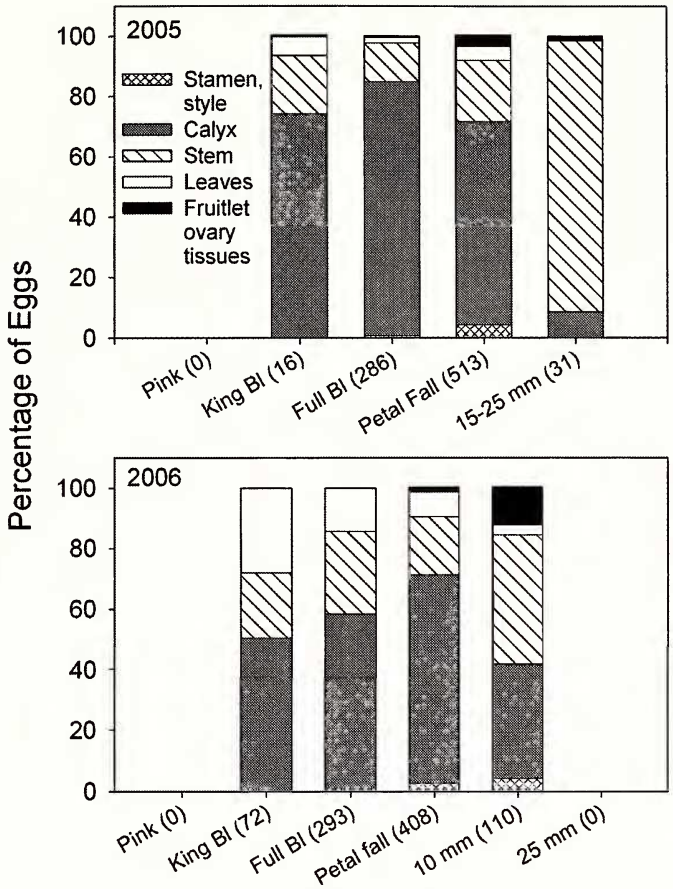

Figure 3. Percentage of eggs deposited in various flower and fruit parts as a function of blossom and fruit stage (2005-06). Numbers in parentheses are total eggs counted in the subsamples.

lent to multiple applications made over the 39-d period when adults were present in blossom clusters.

2006 Insecticide Timing Trial. Fruit damage was affected by timing of spray application (F = 5.74; df = 5,36; P = 0.0005), but not by the type of insecticide used (F = 0.05; df = 1,36; P = 0.83). The interaction term was not significant (F = 0.34, df = 5,36; P = 0.89), thus the main effects means are of most interest (Table 2). Fruit injury on trees treated at full bloom or the end of petal fall (5.7 mm diameter king fruit) was significantly lower than injury on trees treated at all other times (Table 2). Mean percentage of fruit with pansy spot on trees treated at king bloom or any time after 5.7 mm diameter king fruit varied between 3.1 and 3.8% (Table 2), compared to a mean damage of 2.6% in the unsprayed check (data for check plots not shown). Thus, insecticides applied at king bloom or at any interval after the fruit had attained a diameter of 5.7 mm provided no benefit.

In summary, data reported here support a revision of the recommended spray timing for managing western flower thrips in central Washington apple orchards. The current recommendation that insecticides should be applied at pink (Smith *et al.* 2005) is clearly too early for preventing damage, although it may reduce adult thrips populations. There is an optimal spray window from full bloom to 5 mm fruit diameter, similar to that described by Madsen and Jack (1966) for British Columbia, Canada, but later than that described by Terry (1991) for Arizona, USA. The period of optimal timing extended from 8 - 13 d in 2004 - 2006, giving some flexibility in orchard operations. The

Table 1.

Mean percentage of fruit (SEM) with pansy spot from apple tree branches treated with formetanate hydrochloride on different blossom or fruit growth stages, 2004

Stage treated	Application date	% damage[1]
Tight cluster	5 April	11.4 (3.0)ab
Pink	9 April	20.1 (4.4)a
King bloom	13 April	10.3 (3.7)abc
Full bloom	16 April	7.0 (2.5)bcd
Petal fall, 2.7 (0.15) mm	20 April	2.5 (1.7)cde
Petal fall, 2.9 (0.18) mm	23 April	1.7 (1.7)de
Petal fall, 5.6 (0.31) mm	27 April	0.0 (0.0)e
10.9 (0.78) mm	4 May	12.5 (4.3)ab
15.8 (0.61) mm	11 May	11.8 (4.7)abc
All stages	All dates	0.0 (0.0)e

[1] Means followed by the same letter are not significantly different, Least Significant Difference test, α=0.05.

Table 2.

Mean percentage of fruit (SEM) with pansy spot from apple trees treated with either formetanate hydrochloride or spinosad on different blossom or fruit growth stages, 2006[1]

Stage treated	Application date	Formetanate HCl (1 kg AI/ha)	Spinosad (0.14 kg AI/ha)	Pooled means
King bloom	28 April	3.5 (1.8)	2.8 (1.4)	3.1 (1.1)a
Full bloom	4 May	0.5 (0.3)	0.7 (0.5)	0.6 (0.3)b
Petal fall, 5.7 mm	12 May	0.6 (0.4)	1.0 (0.2)	0.8 (0.2)b
12.8 mm	17 May	4.1 (1.9)	3.3 (0.9)	3.7 (1.0)a
17.3 mm	25 May	4.2 (1.1)	3.4 (0.9)	3.8 (0.7)a
26.7 mm	1 June	3.4 (0.7)	3.1 (1.2)	3.3 (0.6)a
Pooled means		2.7 (0.5)a	2.4 (0.4)a	

[1] Means within columns (dates) or between columns (insecticide) followed by the same letter are not significantly different, Least Significant Difference test, α =0.05.

beginning of this period (full bloom) coincides with the timing of blossom thinning sprays, suggesting the possibility of combining materials. The latter part of the spray window coincides with early fruit thinning sprays that are applied at 80% petal fall, or 3-5 mm fruit diameter (Smith *et al.* 2005). A petal-fall or post-petal-fall spray, which would be after bee hives had been removed from the orchard, would minimize damage potential to bees and eliminate the need to apply insecticides in the late evening or at night. The latter timing also opens up the potential use of materials that are effective on thrips, but could not otherwise be used because of bee hazard.

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